

CLAIM AMENDMENTS

The following listing of claims will replace all prior versions and listings of claims in the instant application:

1-28. (Canceled)

29. (Currently Amended) An immunoassay for screening modulators of threonine or serine kinase activity comprising:

a) providing a threonine or serine kinase substrate protein or peptide comprising the sequence motif

-Z-X-Y or -Y-X-Z-

wherein

Z = threonine or serine

X = a sequence of amino acids-in the range of between 1 and 1000 amino acids, which may be the same or different

Y = phospho-tyrosine, phospho-threonine or phospho-serine;

b) adding a test compound;

c) incubating the protein or peptide with a phosphate donor and a threonine or serine kinase under conditions and for a sufficient time to permit phosphorylation of the Z position of the kinase substrate peptide or

protein, wherein the kinase substrate may be a labeled or unlabeled compound;

d) adding an antibody having a specificity to the kinase substrate peptide or protein which is phosphorylated at the Y and Z positions, wherein the antibody may be a labeled or unlabeled antibody;

e) detecting the threonine or serine kinase activity; and

f) comparing the threonine or serine kinase activity in the presence of the test compound with the threonine or serine kinase activity in the absence of the test compound,

wherein altered threonine or serine kinase activity, detected by either labeled antibody or labeled kinase substrate, in the presence of the test compound relative to threonine or serine kinase activity in the absence of the test compound indicates a modulator of threonine or serine kinase activity.

30. (Currently amended) The ~~method~~ immunoassay of claim 29, wherein a threonine or serine kinase inhibitor is indicated by lower threonine or serine kinase activity in the presence of the test compound relative to the threonine or serine kinase activity in the absence of the test compound.

31-35. (Canceled)

36. (Previously presented) The immunoassay according to claim 29, wherein the phosphate donor is ATP, GTP, or a synthetic cosubstrate.

37. (Previously presented) The immunoassay according to claim 29, wherein the immunoassay is performed as a direct binding immunoassay.
38. (Previously presented) The immunoassay according to claim 37, wherein said peptide or protein further comprises a molecular label.
39. (Previously presented) The immunoassay according to claim 37, wherein said antibody further comprises a molecular label.
40. (Previously presented) The immunoassay according to claim 38, wherein said label is selected from the group consisting of a luminescent tag, a radioactive marker, a reporter enzyme, and an affinity ligand.
41. (Previously presented) The immunoassay according to claim 29, wherein the immunoassay is performed as an indirect binding immunoassay.
42. (Previously presented) The immunoassay according to claim 41 further comprising:

g) adding a competitor protein or competitor peptide comprising the sequence motif

-Z'-X'-Y' or -Y'-X'-Z'-

wherein

Z' = phospho-threonine or phospho-serine

X' = a sequence of amino acids, preferably between 1 and 1000 amino acids, which may be the same or different

Y' = phospho-tyrosine, phospho-threonine or phospho-serine.

43. (Previously presented) The immunoassay according to claim 42, wherein the competitor protein or competitor peptide further comprises a label selected from the group consisting of a luminescent tag, a radioactive marker, a reporter enzyme, and an affinity tag.

44. (Currently amended) The immunoassay according to claim 43, wherein the competitor protein or competitor peptide comprises the amino acid sequence of SEQ ID NO:3 such that said sequence motif is

-Y'-X'-Z'-

wherein

Y' is phosphorylated Tyr⁵ at position 5 of SEQ ID NO:3,

X' is Pro⁶ at position 6 of SEQ ID NO:3, and

Z' is phosphorylated Thr⁷ at position 7 of SEQ ID NO:3.

45. (Previously presented) The immunoassay according to claim 29, wherein said e) detecting threonine or serine kinase activity is achieved by fluorescence detection, fluorescence polarization analysis, fluorescence correlation spectroscopy, fluorescence resonance energy transfer analysis, or fluorescence intensity distribution analysis.

46. (Previously presented) The immunoassay according to claim 29, wherein the threonine or serine kinase is a threonine kinase.
47. (Currently Amended) The immunoassay according to claim 29, wherein the substrate protein or substrate peptide comprises the amino acid sequence of SEQ ID NO:2 such that said sequence motif is

-Y-X-Z-

wherein

Y is phosphorylated Tyr⁵ at position 5 of SEQ ID NO:2,

X is Pro⁶ at position 6 of SEQ ID NO:2, and

Z is Thr⁷ at position 7 of SEQ ID NO:2.

48. (Currently amended) An immunoassay for screening modulators of threonine or serine kinase activity comprising:

- a) providing a threonine or serine kinase substrate protein or peptide comprising the sequence motif

-Z-X-Y- or -Y-X-Z-

wherein

Z = threonine or serine

X = a sequence of amino acids-in the range of between 1 and 1000
amino acids, which may be the same or different

Y = phospho-tyrosine, phospho-threonine or phospho-serine;

- b) adding a test compound;
- c) incubating the protein or peptide with a phosphate donor and a threonine or serine kinase under conditions and for a sufficient time to permit phosphorylation of the Z position of the kinase substrate peptide or protein, wherein the kinase substrate may be a labeled or unlabeled compound;
- d) adding an antibody having a specificity to the kinase substrate peptide or protein which is phosphorylated at the Y and Z positions, wherein the antibody may be a labeled or unlabeled antibody;
- e) detecting the threonine or serine kinase activity; and
- f) comparing the threonine or serine kinase activity in the presence of the test compound with the threonine or serine kinase activity in the presence of a known modulator,

wherein altered threonine or serine kinase activity, detected by either labeled antibody or labeled kinase substrate, in the presence of the test compound ~~that is~~ substantially the same as relative to the threonine or serine kinase activity in the

presence of the known modulator indicates a modulator of threonine or serine kinase activity.

49. (New) An immunoassay for screening modulators of threonine or serine kinase activity comprising:

- a) providing a threonine or serine kinase substrate protein or peptide comprising the sequence motif

-Z-X-Y- or -Y-X-Z-

wherein

Z = threonine or serine

X = a sequence of amino acids in the range of between 1 and 1000 amino acids, which may be the same or different

Y = phospho-tyrosine, phospho-threonine or phospho-serine;

- b) adding a test compound;
- c) incubating the protein or peptide with a phosphate donor and a threonine or serine kinase under conditions and for a sufficient time to permit phosphorylation of the Z position of the kinase substrate protein or peptide;
- d) adding an antibody having a specificity to the kinase substrate protein or peptide which is phosphorylated at the Y and Z position;
- e) adding a competitor protein or competitor peptide comprising the sequence motif

-Z'-X'-Y'- or -Y'-X'-Z'-

wherein

Z' = phospho-threonine or phospho-serine

X' = a sequence of amino acids in the range of between 1
and 1000 amino acids, which may be the same or different

Y' = phospho-tyrosine, phospho-threonine or phospho-
serine,

and wherein the competitor protein or competitor peptide is labelled;

- f) detecting the threonine or serine kinase activity; and
- g) comparing the threonine or serine kinase activity in the presence of the test compound with the threonine or serine kinase activity in the absence of the test compound,

wherein altered threonine or serine kinase activity, detected by labelled competitor protein or competitor peptide, in the presence of the test compound relative to threonine or serine kinase activity in the absence of the test compound indicates a modulator of threonine or serine kinase activity.

- 50. (New) The immunoassay according to claim 49, wherein a threonine or serine kinase inhibitor is indicated by lower threonine or serine kinase activity in the presence of the test compound relative to the threonine or serine kinase activity in the absence of the test compound.
- 51. (New) The immunoassay according to claim 49, wherein the phosphate donor is ATP, GTP, or a synthetic cosubstrate.

52. (New) The immunoassay according to claim 49, wherein a label of the competitor protein or competitor peptide is selected from the group consisting of a luminescent tag, a radioactive marker, a reporter enzyme, and an affinity ligand.
53. (New) The immunoassay according to claim 52, wherein the competitor protein or competitor peptide comprises the amino acid sequence of SEQ ID NO:2 such that said sequence motif is -Y'-X'-Z'-
wherein
Y' is phosphorylated Tyr at position 5 of SEQ ID NO:2,
X' is Pro at position 6 of SEQ ID NO:2, and
Z' is phosphorylated Thr at position 7 of SEQ ID NO:2.
54. (New) The immunoassay according to claim 52, wherein the competitor protein or competitor peptide comprises the amino acid sequence of SEQ ID NO:3 such that said sequence motif is -Y'-X'-Z'-
wherein
Y' is phosphorylated Tyr at position 5 of SEQ ID NO:3,
X' is Pro at position 6 of SEQ ID NO:3, and
Z' is phosphorylated Thr at position 7 of SEQ ID NO:3.
55. (New) The immunoassay according to claim 49, wherein said f) detecting threonine or serine kinase activity is achieved by fluorescence detection, fluorescence polarization analysis, fluorescence correlation analysis, fluorescence resonance energy transfer analysis, or fluorescence intensity distribution analysis.

56. (New) The immunoassay according to claim 49, wherein the threonine or serine kinase is a threonine kinase.
57. (New) The immunoassay according to claim 49, wherein the kinase substrate protein or peptide comprises the amino acid sequence of SEQ ID NO:2 such that said sequence motif is -Y-X-Z-
wherein
Y is phosphorylated Tyr at position 5 of SEQ ID NO:2,
X is Pro at position 6 of SEQ ID NO:2, and
Z is Thr at position 7 of SEQ ID NO:2.
58. (New) The immunoassay according to claim 49, wherein the kinase substrate protein or peptide comprises the amino acid sequence of SEQ ID NO:3 such that said sequence motif is -Y-X-Z-
wherein
Y is phosphorylated Tyr at position 5 of SEQ ID NO:3,
X is Pro at position 6 of SEQ ID NO:3, and
Z is Thr at position 7 of SEQ ID NO:3.
59. (New) An immunoassay for screening modulators of threonine or serine kinase activity comprising:
- a) providing a threonine or serine kinase substrate protein or peptide comprising the sequence motif
-Z-X-Y- or -Y-X-Z-
wherein

Z = threonine or serine

X = a sequence of amino acids in the range of between 1 and 1000 amino acids,
which may be the same or different

Y = phospho-tyrosine, phospho-threonine or phospho-serine;

- b) adding a test compound;
- c) incubating the protein or peptide with a phosphate donor and a threonine or serine kinase under conditions and for a sufficient time to permit phosphorylation of the Z position of the kinase substrate protein or peptide;
- d) adding an antibody having a specificity to the kinase substrate protein or peptide which is phosphorylated at the Y and Z position;
- e) adding a competitor protein or competitor peptide comprising the sequence motif

-Z'-X'-Y'- or -Y'-X'-Z'-

wherein

Z' = phospho-threonine or phospho-serine

X' = a sequence of amino acids in the range of between 1 and 1000 amino acids,
which may be the same or different

Y' = phospho-tyrosine, phospho-threonine or phospho-serine,

and wherein the competitor protein or competitor peptide is labelled;

- f) detecting the threonine or serine kinase activity; and
- g) comparing the threonine or serine kinase activity in the presence of the test compound with the threonine or serine kinase activity in the presence of a known modulator,

wherein threonine or serine kinase activity, detected by labelled competitor protein or competitor peptide, in the presence of the test compound as relative to the threonine or serine kinase activity in the presence of the known modulator indicates a modulator of threonine or serine kinase activity.

60. (New) The immunoassay according to claim 59, wherein the phosphate donor is ATP, GTP, or a synthetic cosubstrate.
61. (New) The immunoassay according to claim 59, wherein the label of the competitor protein or competitor peptide is selected from the group consisting of a luminescent tag, a radioactive marker, a reporter enzyme, and an affinity ligand.
62. (New) The immunoassay according to claim 61 wherein the competitor protein or competitor peptide comprises the amino acid sequence of SEQ ID NO:2 such that said sequence motif is -Y'-X'-Z'-
wherein
Y' is phosphorylated Tyr at position 5 of SEQ ID NO:2,
X' is Pro at position 6 of SEQ ID NO:2, and
Z' is phosphorylated Thr at position 7 of SEQ ID NO:2.
63. (New) The immunoassay according to claim 61 wherein the competitor protein or competitor peptide comprises the amino acid sequence of SEQ ID NO:3 such that said sequence motif is -Y'-X'-Z'-
wherein
Y' is phosphorylated Tyr at position 5 of SEQ ID NO:3,
X' is Pro at position 6 of SEQ ID NO:3, and
Z' is phosphorylated Thr at position 7 of SEQ ID NO:3.

64. (New) The immunoassay according to claim 59, wherein said f) detecting threonine or serine kinase activity is achieved by fluorescence detection, fluorescence polarization analysis, fluorescence correlation analysis, fluorescence resonance energy transfer analysis, or fluorescence intensity distribution analysis.
65. (New) The immunoassay according to claim 59, wherein the threonine or serine kinase is a threonine kinase.
66. (New) The immunoassay according to claim 59, wherein the kinase substrate protein or peptide comprises the amino acid sequence of SEQ ID NO:2 such that said sequence motif is -Y-X-Z-
wherein
Y is phosphorylated Tyr at position 5 of SEQ ID NO:2,
X is Pro at position 6 of SEQ ID NO:2, and
Z is Thr at position 7 of SEQ ID NO:2.
67. (New) The immunoassay according to claim 59, wherein the kinase substrate protein or peptide comprises the amino acid sequence of SEQ ID NO:3 such that said sequence motif is -Y-X-Z-
wherein
Y is phosphorylated Tyr at position 5 of SEQ ID NO:3,
X is Pro at position 6 of SEQ ID NO:3, and
Z is Thr at position 7 of SEQ ID NO:3.